ON THE CONDUCTION OF THE CORTICAL CHANGE AT FERTILIZATION IN THE STARFISH EGG

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A number of radical changes take place in the echinoderm egg when it is fertilized, one of which is a change in the cortex which prevents the entrance of a second sperm. That the egg is not entirely passive at the time of the entrance of the sperm is well known in a number of eggs from the phenomenon of the entrance cone which flows out and partly engulfs the sperm head.

For many years there has been a difference of opinion as to whether the fertilization membrane pre-exists in the sea urchin egg before fertilization. The more recent general opinion has been that some sort of a membrane is present. A. R. Moore (1929), however, has recently presented evidence which he regards as supporting Loeb's view that this membrane does not pre-exist but is formed *de novo* at the time of fertilization. In the case of the starfish egg, however, there is no question that a definite tough membrane pre-exists which normally lifts off following fertilization. Chambers (1921) has lifted this membrane from the unfertilized *Asterias* egg with a micro-needle. It has also been shown (Whitaker, 1928) that the cytoplasm of the egg of the starfish *Patiria miniata* may be readily divided into two separate parts with a micro-needle without severing the tough outermost membrane. If an egg which is divided in this way is inseminated, both fragments can be fertilized and will develop independently within the common fertilization membrane. In this case there can be no doubt that a morphological membrane is present, and that it lifts off as the fertilization membrane, even though the properties of the membrane may perhaps change following fertilization.

As early as 1878, O. Hertwig expressed the view that it is the egg plasma itself rather than the fertilization membrane which can prevent the entrance of a second sperm. Just (1919) has pointed out that obviously the fertilization membrane rises off the egg so late after the contact of the fertilizing sperm that another and more rapid change of the cortex must be postulated to explain rejection of the second sperm.[1]

[1] In *Arbacia* the membrane begins to lift off about eighteen seconds after contact of the fertilizing sperm, and in *Echinarachnius* after about twenty to forty seconds (from Just, 1929).

Just has further observed in *Echinarachnius* that this change, which he calls a " wave of negativity," spreads rapidly from the point of contact of the first sperm, just as the lifting of the fertilization membrane itself spreads from this point, but spreading much more rapidly so that it has covered the entire egg before the fertilization membrane has even started to rise at the entrance point of the sperm. His evidence for this " wave of negativity " is based on observations on living eggs. As soon as the tip of the first sperm has penetrated the egg, other sperm are not engulfed in the immediate vicinity of the first sperm, although they may be taken in further around the egg. As the first sperm penetrates still further, the " wave of negativity " progresses further around the egg until at the moment the sperm head has entirely disappeared into the egg, only the opposite pole of the egg can engulf another sperm. Sperm which become attached but do not enter the egg are lifted off as the fertilization membrane rises.

More recently Just (1929) and others have observed that in eggs which are in excellent condition, the cortical reaction is so rapid as to be practically instantaneous. It is practically impossible to produce polyspermy by the use of concentrated sperm suspensions. It is evident that in such innoculations the time elapsing between the contact of the first and the contact of the second sperm must be an exceedingly small fraction of a second. The spread of the cortical change in eggs in perfect condition is much too rapid to be traced by the eye. Just (1930, page 337) says, " At least for the normally monospermic ova of the marine forms which I have studied, it is certainly true that if they are in optimum condition, polyspermy is difficult if not impossible. Such ova in order to become polyspermic must undergo treatment which impairs their cortices. . . . Doubtless the very instant that contact between spermatozoon and ova is made, polyspermy is blocked."

The response of the egg to the stimulus of the first sperm involves the whole interior of the egg as well as the cortex. Flowing of the interior protoplasm immediately succeeds fertilization, as well as changes in viscosity, etc. (Heilbrunn, 1928). The interior changes may be secondary, however,—that is, consequences of changes in permeability and surface tension of the cortical region.

That the cortex performs a necessary function in admitting the first sperm, as well as in preventing polyspermy, has been shown by Just (1923) for *Echinarachnius* and for *Arbacia* and by Chambers (1921) for the starfish *Asterias*. Just burst eggs by returning them from hypertonic to normal sea water, and also by passing eggs through fine-meshed bolting silk and through lens paper to obtain endoplasmic buds or fragments bearing none of the original cortex of the egg. Chambers obtained similar fragments by means of the microdissection needle. In none of these eggs did the endoplasmic buds react to sperm or become fertilized.

The present observations add little evidence as to the nature of the cortical reaction. They are concerned with the locus or site of conduction of the spreading impulse. If it starts at a point on the cortex, it must spread either in the form of the cortical change itself, or else it must be an equilibrium shift transmitted either around the cortex, or through the egg, giving rise to the cortical change.

In a previous paper on the development of fragments of *Patiria* eggs (Whitaker, 1928), 69 cases are recorded in which two completely separated fragments lying within the same fertilization membrane-to-be were inseminated and became fertilized independently, one sperm entering each fragment. This result is almost invariably obtained when the two masses of cytoplasm are not in contact at the time of insemination. This seems to show that the impulse which spreads through the egg and results in the cortical change which prevents the entrance of a second sperm does not pass through this outermost membrane. There is no evidence of injury to the outer membrane as a result of pinching the protoplasm. It lifts off in the normal way after fertilization, although sometimes it is temporarily constricted where the needle has pressed. Additional experiments have been made in which eggs have been pinched into two cytoplasmic fragments lying within a common outer membrane, and the fragments have been allowed to flow together before insemination. In this case the treatment of the outer membrane is practically identical with that in the cases of fragments which remain apart, the difference between the two types of cases being only in the fusion or non-fusion of the protoplasm within the membrane. Only sets of eggs were used in which control tests gave 98–100 per cent fertilization. A moderately concentrated suspension of sperm was used. As a control, to test for polyspermy, five or six normal eggs were placed by means of a mouth pipette around the experimental egg, not more than several egg diameters away. Half an hour after insemination the experimental egg was transferred to a separate dish, since at the time of cleavage when the eggs are changing shape there is otherwise some danger of mistaken identity. For some time after insemination, however, the marks of cutting are clearly discernible on the experimental egg. In all cases the eggs were left in fresh sea water for about ten minutes after cutting before insemination. It has been found in a number of echinoderm eggs that this procedure permits the fragments to recover from the operation and in the case of *Arbacia* eggs, for example, it avoids polyspermy which often results from immediate insemination.

The degree of separation of two cytoplasmic fragments lying within the same outer membrane can be regulated by the extent to which the membrane is stretched with the needle, a very slight difference in the amount of stretching determining whether the fragments will flow back together or not. The eggs of *Patiria* are large, averaging about 190 or 200 microns in diameter, and they are comparatively clear, so that the grosser aspects of the nuclear and astral phenomena can usually be clearly seen in the living egg or egg fragment.

Eleven eggs were cut, after the fashion of the 69 cases already quoted, so that the two fragments were separate at the time of insemination. The control eggs lying near the experimental egg showed no polyspermy. In 9 of the 11 cases, both fragments were fertilized independently, each dividing with a single amphiaster into two normal cells (Fig. 1, *a, b, c*). In two cases one fragment only became fertilized with one sperm, the other fragment failing to become fertilized.

Twenty-five eggs were cut into two fragments and allowed to flow completely together before insemination. In 18 of these cases the fragments remained separate for one to two seconds, in three cases for more than five seconds, and in four cases for fifteen seconds to one minute. If the fragments remain apart for several minutes, a solidification of the cut surface usually prevents a complete refusion of the fragments, or in cases of fusion results in an ectoplasmic wall along the surface of fusion. In the 25 cases of complete fusion, 23 cleaved with one amphiaster into a normal two-cell stage. Apparently only one sperm had entered. There were no cases of polyspermy in the control eggs. In two cases division was by means of a triaster into three cells, apparently due to the entrance of two sperm. One of these cases of polyspermy was an egg separated for one to two seconds. The controls showed no polyspermy. The other was an egg which had been apart for more than fifteen seconds. The controls in this last case showed polyspermy, probably indicating that insemination was too heavy and that the eggs were not in the best condition.

The results show that when a sperm enters one part of an egg, usually no other sperm enters any other part of the egg which is joined by protoplasmic fusion, but another sperm does enter a part of the egg which has only the same outer membrane in common, equally well as if the parts of the egg were entirely separated and removed from one another. Apparently the cortical change which normally prevents polyspermy is not conducted through the outermost membrane, the fertilization membrane-to-be. It is conducted through the protoplasm within this membrane.

The question naturally arises: To what extent must two fragments be associated in order that the fertilization of one shall prevent the fertilization of the other? Is mere contact of the surfaces of two fragments within the outer membrane sufficient or is fusion necessary? In contrast with our extreme cases of complete separation and complete fusion, which can be identified with certainty and in which the results are clear cut, it is difficult to tell by observation in the border line cases in which the two fragments are in contact precisely what degree of fusion has taken place. Fragments which are in contact but not fused at the time of insemination usually fuse soon after fertilization. Although no statement can be made at present as to the minimum degree of contact or fusion necessary to transmit the impulse, the following experiments throw some light on the question.

Fifteen eggs were cut into two fragments which remained separate for more than a minute and then came together so that at the time of insemination they are classed as (1) touching, (2) in good contact, or (3) fused at a narrow neck, the neck being from less than ten to thirty microns through. The eggs are 200 microns in diameter. In five cases the fragments were touching. In four of these cases one sperm entered each fragment. The fragments subsequently fused, but retained an ectoplasmic partition and separated at the time of cleavage, each com-

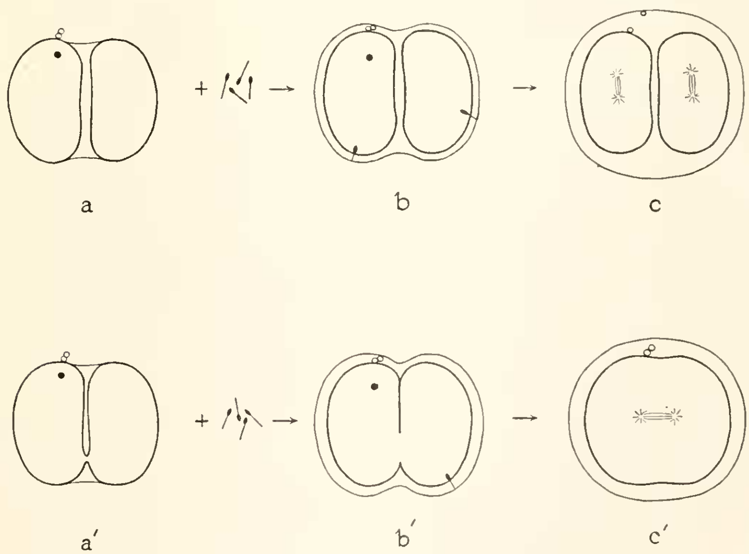

Fig. 1. Diagrams before insemination, after insemination, and just before cleavage, of eggs cut without severing the outermost membrane. (Sperm disproportionately enlarged.)

a, b, c. An egg with halves separated at time of insemination.

a′, b′, c′. An egg with halves joined by a narrow neck at time of insemination.

ponent dividing with a single amphiaster into two cells. The fifth case admitted only one sperm and divided as a whole into two cells. In three cases the fragments were in good contact, that is, about on the border between touching and slightly fused. Two of these cases had one amphiaster and divided into two cells. The third admitted a sperm into each half and divided directly into four cells. In seven cases the two halves were truly fused by a narrow neck at one end of the cut. Shortly after fertilization more complete fusion took place. In all of these seven cases only one sperm entered the joined fragments. In four of the cases normal division into two cells followed (Fig. 1, *a′*, *b′*, *c′*). In two cases the nucleus of the sperm, which had entered the non-nucleated fragment of the egg, was not able to pass through the gelated partition of the fused surfaces to fuse with the egg nucleus, and only the fragment containing the sperm nucleus divided into a normal two-cell stage. The other half separated off at the time of division but did not itself divide. In the seventh of these cases the sperm entered the non-nucleated fragment, which divided, and the nucleated fragment which apparently received no sperm divided late, involving the egg nucleus only.

These results on fragments which are only in slight contact show that a very narrow neck of truly fused protoplasm conducts the impulse. Mere contact without fusion probably does not.

Summary

When eggs of the starfish are pinched into two cytoplasmic fragments which lie within the same outermost membrane (the fertilization membrane-to-be), and are inseminated while the two fragments are separate, each fragment receives one sperm. If the fragments completely fuse before insemination, only one sperm enters the fused egg. If the fragments are merely touching, each may receive a sperm. If they are joined by a very narrow fused neck of protoplasm, only one sperm enters.

Conclusion

The outermost membrane of the starfish egg (the fertilization membrane-to-be) does not conduct the cortical change at fertilization which prevents the entrance of a second sperm. This change is conducted by the protoplasm within the membrane.

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